

Interaction of a tryptophan-containing peptide with chromatin core particles

A fluorescence study

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The binding of a tetrapeptide lysyltryptophylglycyllysine to nucleosome core particles has been investigated using UV absorption and fluorescence spectroscopy. Modifications of the absorption spectra and fluorescence quenching of the tryptophyl residue are consistent with stacking between the indole ring and nucleic acid bases. Therefore DNA interactions with histones do not prevent stacking of the tryptophyl residue with nucleic acid bases in the peptide–core particle complexes. The number of peptide binding sites is reduced to half that of naked DNA.

Peptide–DNA complex

Stacking interaction

Core particle

Fluorescence

1. INTRODUCTION

Model studies have shown that stacking interactions between aromatic amino acids and nucleic acid bases provide basic oligopeptides with a binding specificity toward single-stranded polynucleotides [1–3]. Stacking interactions involving tryptophyl, tyrosyl or phenylalanyl residues have been suggested in complexes between gene 5 protein from phage fd [4,7] or gene 32 protein from phage T4 [5,6] and single-stranded nucleic acids. Such interactions could play a role in the selective recognition of nucleic acids by proteins (review [7,8,22,23]).

In eukaryotic cells DNA is folded around histone cores in nucleosome particles. The association of histones with DNA is essentially established through electrostatic interactions between positively charged amino acids and phosphate groups

[9,10]. The formation of stacked complexes between aromatic amino acids and nucleic acid bases in native DNA induces a local distortion of the double helix. The association of histones with DNA could therefore prevent the formation of such complexes in the regions of DNA which are wrapped around the histone core. In fact, interactions of intercalating drugs such as ethidium bromide has been shown to be different in chromatin as compared with naked DNA [11]. The DNA segments linking nucleosomes together are privileged binding sites.

We report here the results of a fluorescence study of the association between a tetrapeptide [lysyltryptophylglycyllysine *O*-(*tert*-butyl) ester, KWGKtBu] and nucleosome core particles.

2. MATERIALS AND METHODS

The peptides KWGKtBu and lysyltryptophyllysine (KWK) were purchased from Bachem and E.

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coli DNA from Sigma. Denaturation was carried out by heating DNA samples in sealed tubes at 100°C for 15 min and rapid cooling in ice.

Nucleosome core particles were prepared by micrococcal nuclease digestion of chicken erythrocyte chromatin which had been stripped of histones H₁ and H₅, as in [12]. Analysis of deproteinized core particles by electrophoresis on acrylamide-formamide gels gave a single band corresponding to DNA fragments 145 base-pairs long [13].

Absorbance spectra and melting curves were recorded with a Uvikon 820 spectrophotometer. Fluorescence measurements were performed with a Fica 55 differential spectrofluorometer which is automatically corrected for excitation fluctuations. Polarization measurements were performed on the Fica fluorometer using Glan-Thomson polarizers.

Unless otherwise specified, solutions of peptides and DNA were prepared in 1 mM sodium cacodylate buffer (pH 6.0) which contained 1 mM NaCl and 0.2 mM EDTA. DNA and core particles were extensively dialyzed against the above buffer prior to use. Concentration of peptide, DNA and core particle solutions were determined spectrophotometrically using the following molar extinction coefficients: 5600 M⁻¹·cm⁻¹ at 280 nm for KWGKtBu and KWK and 6500 M⁻¹·cm⁻¹ at 260 nm for *E. coli* DNA and core particles.

3. RESULTS AND DISCUSSION

3.1. Absorbance measurements

Binding of KWGKtBu to DNA results in modification of the absorbance spectra of the interacting molecules. Difference absorption spectra exhibit a positive band at wavelengths longer than 290 nm and a negative band centered at about 260 nm (fig.1). The effect is larger with denatured than with native DNA. Such difference spectra have been observed for the complexes KWK–DNA and the two bands attributed to the formation of a stacked complex which involves the indole ring of tryptophan and nucleic acid bases [2,14]. The negative band exhibits shoulders around 280 nm which are characteristic of a perturbation of the environment of the tryptophyl ring. The negative contribution near 260 nm probably reflects the nucleic acid absorption.

As shown in fig.1 addition of KWGKtBu to

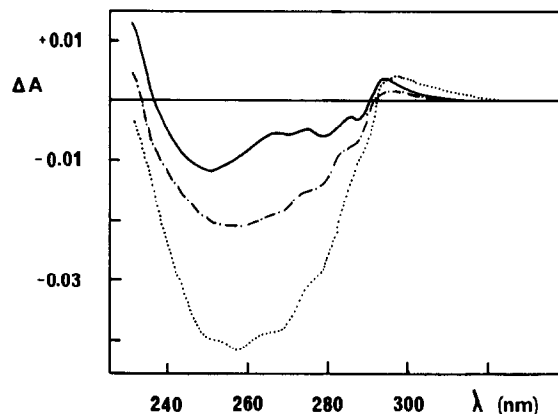
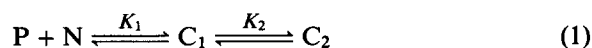


Fig.1. Difference absorption spectra of 2.54×10^{-5} M KWGKtBu in the presence of 1.60×10^{-4} M native *E. coli* DNA (—), erythrocyte chromatin core particles (---), or heat-denatured *E. coli* DNA (···). Measurements were made in 1 mM Na cacodylate buffer (pH 6.0) containing 1 mM NaCl and 0.2 mM EDTA.

nucleosome core particles leads to an increase of the absorbance at $\lambda > 290$ nm and to the appearance of a negative band at 260 nm. The amplitude of these bands increases with peptide concentration for a given core particle concentration. The amplitude of the negative band increases in the order: native DNA < core particles < heat-denatured DNA. This difference spectrum is not observed at high ionic strength, i.e., under conditions which lead to complex dissociation. These results indicate that KWGKtBu binds to nucleosome core particles and suggest that the interactions involved in this association are similar to those observed in KWK–DNA complexes.

3.2. Binding of KWGKtBu to native and heat-denatured DNA

The following two step-model (eq.1) has been proposed for the binding of KWK to polynucleotides:



where P represents the peptide and N the nucleic acid. Only electrostatic interactions are involved in the formation of complex C₁. Besides electrostatic interactions the formation of complex C₂ involves stacking between the indole ring and nucleic acid bases. Peptide molecules in complex C₁ have the

same fluorescence quantum yield as the free molecules whereas the fluorescence emission of the peptide in complex C_2 is totally quenched ($\phi_F = 0$) [15]. A more complete scheme involving different conformers of the peptide has been proposed [16] on the basis of fluorescence lifetime measurements. However the scheme given in eq.1 remains a good approximation especially in the case of native and heat-denatured DNA.

The binding of KWGKtBu to DNA leads to partial quenching of its fluorescence. This effect is reversed upon addition of NaCl. These results and those obtained from absorption measurements (see above and [1,14]) suggest that both electrostatic and stacking interactions are involved in the association between this peptide and DNA. The average fluorescence lifetime of the peptide is not strongly affected in the presence of DNA [16]. Therefore at low ratios of peptide bound to DNA the results can be analyzed according to the following relationship (see [1]):

$$R = \frac{I_F}{I_F - I_L} = 1 + \frac{1}{K_2} + \frac{1}{K_1 K_2} = \frac{1}{N_0} \quad (2)$$

where I_F and I_L are the fluorescence intensities of the peptide in the absence and presence of DNA, respectively. The screening effect was taken into account as in [1]. N_0 is the total DNA concentration. Plotting $I_F/(I_F - I_L)$ vs $1/N_0$ should give a straight line whose intercept with the ordinate axis and slope are equal to $1 + 1/K_2$ and $1/K_1 K_2$, respectively.

Analysis of fluorescence data according to eq.2 for the binding of KWGKtBu to native and heat-denatured DNA is shown in fig.2. As previously found with the tripeptide KWK higher values of K_2 were obtained for heat-denatured than for native DNA. This means that the formation of complex C_2 is favoured in single-stranded structures as compared to double-stranded ones. This was ascribed to the fact that stacking of aromatic amino acids with nucleic bases induces a local distortion of the polynucleotide chain and therefore requires less energy in single-stranded than in double-stranded nucleic acids [2]. As expected the value of K_2 does not depend on ionic strength (see [2]). Similar values of K_2 were found for both the tetrapeptide KWGKtBu and the tripeptide KWK: $K_2 = 0.40 \pm 0.05$ and 6 ± 0.5 for native and heat-denatured DNA, respectively ([2] and fig.2).

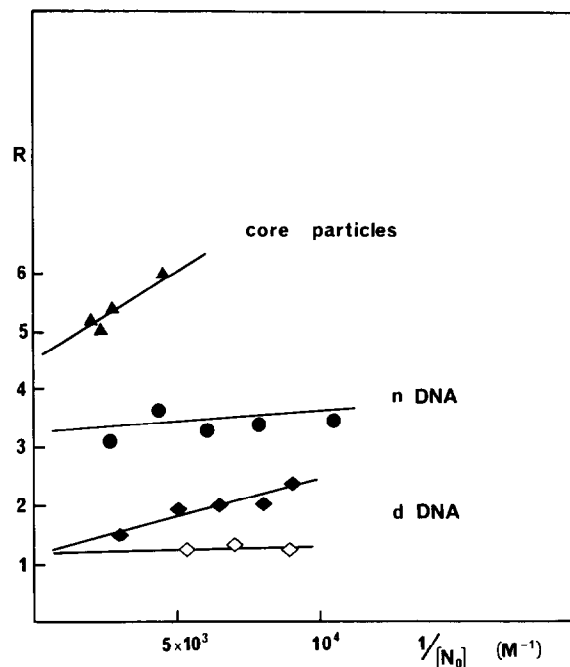


Fig.2. Analysis of fluorescence data according to eq.2 for the binding of KWGKtBu to erythrocyte chromatin core particles (Δ), native (\bullet) and heat-denatured *E. coli* DNA (\diamond, \blacklozenge). Experiments were performed at 5°C in 1 mM Na cacodylate buffer (pH 6.0) containing 0.2 mM EDTA and 1 mM NaCl (\bullet, Δ), 10 mM NaCl (\diamond) or 50 mM NaCl (\blacklozenge).

With denatured DNA the association constant K_1 is one order of magnitude higher for KWGKtBu than for KWK (not shown). This must be ascribed to the overall charge of the peptides. As the binding of these peptides to nucleic acids is mainly governed by electrostatic interactions, blocking the carboxylic group by a *tert*-butyl substituent in KWGKtBu results in increased stability of the complex KWGKtBu–DNA as compared to KWK–DNA.

The weak quenching of the peptide fluorescence did not allow us to measure accurately K_1 for native DNA. However, the same result is expected, i.e., greater electrostatic interactions with KWGKtBu than with KWK. This is confirmed by the higher ionic strength required to dissociate the complexes formed by the tetrapeptide. The ionic strength at which 50% dissociation is observed is 6 and 15 mM for KWK and KWGKtBu, respectively.

3.3. Binding of KWGKtBu to core particles

Fluorescence measurements were carried out to

characterize the binding of KWGKtBu to nucleosome core particles. As observed with native DNA addition of this peptide to core particle solutions results in partial quenching of the tryptophan emission whose amplitude increases with the core particle concentration. This effect is reversed upon addition of NaCl up to 0.5 M. Results analyzed according to eq.2 are shown in fig.2. The K_2 value is only slightly lower than that obtained with native DNA (0.3 and 0.45, respectively). Therefore, the presence of histones prevents neither the binding of the peptide to the DNA chain nor the formation of stacked complexes. However it should be pointed out that the value of K_2 is an average over all possible binding sites. Folding the DNA chain around the histone octamer could lead to the appearance of regions in which the formation of stacked complexes is favoured as compared to naked DNA whereas in other regions the K_2 value could be very low due to strong interactions of histones with DNA.

To determine the relative affinity of the peptide for DNA and core particles, equilibrium dialysis experiments were performed using a 3-compartment cell: the first compartment contained native DNA, the middle one KWGKtBu and the third one core particles at the same DNA concentration as in the first compartment. After equilibrium was reached (at least 6 h) the fluorescence emission of the solution in each compartment was measured before and after addition of concentrated NaCl to dissociate peptide-DNA complexes. By comparison with a solution of peptide of known concentration, the concentration of KWGKtBu in each compartment was determined and the concentration of complexes calculated (screening effect of DNA, dilution and salt effect upon the free peptide emission were taken into account). Results analyzed according to the Scatchard relationship are shown in fig.3. The plots obtained for native DNA and core particles are parallel thus indicating that the association constants are the same in both cases. Equilibrium dialysis measures the overall association constant $K = K_1(1 + K_2)$. The equilibrium constant K_2 determined from fluorescence quenching data is quite similar for native DNA and core particles (see above). Therefore it can be concluded that the association constant K_1 is very similar for native DNA and core particles. The formation of the first type of complex (C_1 , eq.1) is only governed

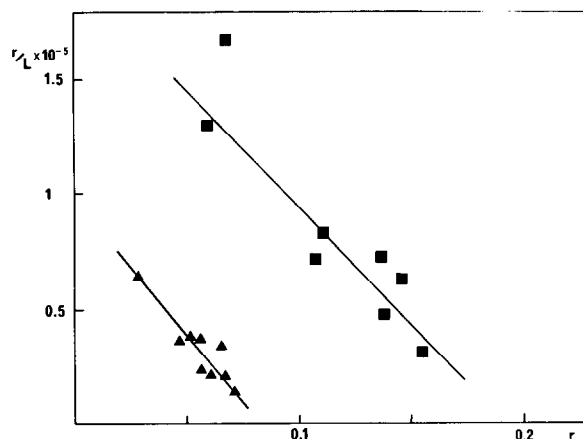


Fig.3. Scatchard plots for the binding of KWGKtBu to native *E. coli* DNA (■) and erythrocyte chromatin core particles (▲) at 5°C in 1 mM Na cacodylate buffer (pH 6.0) containing 1 mM NaCl and 0.2 mM EDTA.

by electrostatic interactions. The above results therefore show that despite the presence of the histone lysine and arginine residues, the electrostatic potential in the vicinity of the phosphate groups involved in the association with the peptide is not very different in the core particles from that of DNA alone.

The intercept of the Scatchard plots with the abscissa indicates that the average number of nucleotides per bound peptide molecule is about twice as large for core particles as for naked DNA. This could reflect the fact that part of the DNA phosphate groups are not available for interaction with the peptide due to the presence of the histone octamer even though the number of phosphate groups involved in direct interactions with histone positive groups is much lower than 145 per nucleosome core. It was previously estimated that only 15–20% of Lys and Arg residues are involved in direct interactions with the DNA backbone [17]. This could also be due to restricted capacity of the DNA in nucleosome cores for accommodating the peptide as compared to naked DNA.

In the two-step model (eq.1), peptide molecules bound to DNA in the first type of complex are expected to have restricted mobility as compared to free molecules. Therefore the fluorescence emission of such complexed molecules should be polarized. In fact the polarization ratio of KWGKtBu bound to oligonucleosomes ($p = 0.05$) is higher than that of the free peptide in the absence or presence of

oligonucleosomes under dissociating conditions ($p = 0.01$). This polarization ratio is similar to that observed with native DNA but is rather low compared to that measured in a viscous medium like glycerol at 5°C ($p = 0.20$) indicating that the indole ring has a high freedom of motion in the electrostatic complex as has been concluded from dynamic measurements with KWGKtBu [15].

3.4. Melting profiles of core particle-KWGKtBu complexes

The melting of DNA in the nucleosome core particle is biphasic, the first denaturation phase corresponding to melting of DNA termini [17,18]. We measured the thermal stability of the nucleosome core particle when complexed with the tetrapeptide KWGKtBu. The melting profiles obtained for various peptide/core particle phosphate ratios are shown in fig.4. At low ratios (<2) the melting curves of the complexes do not differ from that of the free core particles. At a higher ratio (see fig.4) an increase of the cooperativity of the first denaturation phase is observed together with a slight decrease of the midpoint of the second transition. At a ratio higher than 8 the amplitude of the first

transition is increased from 25% to about 80% of the total hyperchromicity suggesting that under these conditions peptide molecules could induce the unfolding of the DNA helix from the histone core. Binding of KWGKtBu to naked DNA increases the melting temperature from 50 to 60°C for peptide/DNA ratios higher than 2.5 under similar ionic conditions (1 mM cacodylate buffer). All the fluorescence measurements reported above were carried out at peptide/phosphate ratios of less than 0.05. The equilibrium dialysis experiment used higher peptide concentrations but the peptide/phosphate ratio was always lower than 0.5. The melting profile of core particles is unaffected under these conditions. Therefore the data reported above reflect the binding of KWGKtBu to intact core particles.

4. CONCLUSIONS

From our results it is apparent that the presence of histones does not prevent the binding of the peptide KWGKtBu to DNA. Moreover, despite the presence of the positive charges of lysine and arginine residues the electrostatic association constant for core particles is not lower than that for binding of the peptide to naked DNA.

We have also demonstrated that the stacking interactions between the indole ring of tryptophan and nucleic acid bases still occur in core particles. We have shown that these interactions could allow tryptophan-containing peptides to recognize locally destabilized regions of DNA induced by UV-irradiation or chemical damage [2,19,20]. Stacking of tyrosyl and phenylalanyl residues with nucleic acid bases has been demonstrated in phage fd gene 5 protein-oligonucleotide complexes [4,7]. It was also suggested that some tyrosyl and tryptophyl residues of the gene 32 protein from phage T4 could be stacked with nucleic acid bases in gene 32 protein-nucleic acid complexes [8,21]. Such interactions could be a general way for single-strand binding proteins to ensure at least part of their specificity. Other proteins or enzymes might also utilize this possibility to bind single-stranded regions in DNA. The present results with core particles suggest that stacking interactions could still allow DNA repair enzymes or proteins to recognize damaged regions or transiently open DNA structures in chromatin.

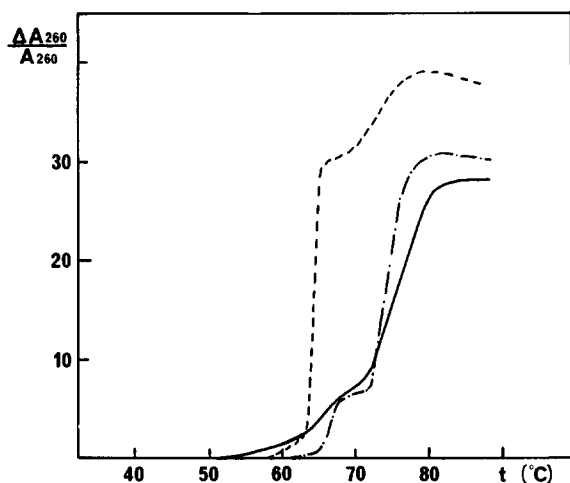


Fig.4. Melting curves of 2.15×10^{-4} M nucleosome core particles alone (—) and in the presence of 8.6×10^{-4} M KWGKtBu (---) and 1.72×10^{-3} M KWGKtBu (-.-.-). Buffer: 1 mM Na cacodylate, 1 mM NaCl, 0.2 mM EDTA (pH 6.0). With 1.05×10^{-4} M KWGKtBu the melting curve cannot be distinguished from that of core particles alone.

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